Section A-Research paper
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A Comparative Analysis of Immuno-Histochemical Expression Patterns of Estrogen Receptor and Human Epidermal Growth Factor Receptor-2 Biomarkers in Breast Cancer

Subtitles: Expression Patterns of Estrogen Receptor and Human Epidermal Growth Factor Receptor-2 Biomarkers in Breast Cancer Tissues

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Abstract

Background/purpose: Breast cancer stands as the most prevalent form of cancer among women, serving as the foremost contributor to cancer-linked fatalities and health issues in the female population. Within the realm of breast cancer, the distinct biologic subgroups are categorized through two vital biomarkers: Estrogen Receptor(ER) alpha and Human Epidermal Growth Factor Receptor(HER) 2. A positive presence of ER- α bears a favorable prognosis. The current investigation seeks to assess the relative immunohistochemical expression of estrogen receptors and HER-2 biomarkers in breast cancer cases. Sixty-two formalin-fixed and paraffin-embedded archived breast tissue blocks were procured from the archive of the histopathology laboratory at AE-FUTHA in Abakaliki.

Methods: The study employed immune-histochemistry staining (IHC), along with Periodic Acid Schiff (PAS) and Hematoxylin and Eosin (H&E) staining, to visualize and demonstrate the characteristics of the breast tissue samples.
Results: The findings predominantly indicated the presence of ductal carcinoma, colloid carcinoma, and Paget disease within invasive ductal carcinoma. The immunohistochemistry results were graded on a scale of 0 to 5 based on intensity. Among the 62 sections of breast cancer tissue examined, 59 (95. 2%) exhibited a negative expression of ER, while a minimal 3 (4. 8%) displayed very weak expression. In contrast, HER2 exhibited strong expression in 7 (11. 3%), moderate expression in 50 (80. 6%), weak expression in 4 (6. 5%), and very weak expression in 1 (1. 6%).
Conclusion: Summarily, this study underscores the prevalence of heightened HER2 expression and diminished ER expression within the examined breast cancer tissues.
Key words: Estrogen Receptor, Human Estrogen Receptor-2, Biomarkers, Breast Cancer, Periodic Acid Schiff, Immunohistochemistry and HER-2

INTRODUCTION

Presently, breast cancer has emerged as the prevailing form of cancer and stands as the primary contributor to fatalities associated with cancer among women. Anticipated to yield a mortality rate of around 30%, the weight of this challenge affects patients, their families, and society at large. A significant portion of these unfortunate outcomes can be traced back to metastatic disease, a condition that frequently acquires resistance against conventional treatment approaches, rendering it challenging to address through current therapeutic methodologies.

Biomarker analysis of invasive breast carcinoma is required for understanding the biology of a patient's disease. Routinely performed tests include immunohistochemistry for ER, progesterone receptor (PR), and Ki67, a proliferation marker, along with either immunohistochemistry and/or fluorescence in situ hybridization (FISH) for HER2 protein overexpression/gene amplification. These biomarkers find widespread application in directing treatment through targeted therapies like hormonal treatments and anti-HER2 agents. Moreover, they serve as proxies for the molecular categorization of breast tumors, a classification with notable prognostic implications (Tang and Tse 2016; Sorlie et al. 2001; Perou et al. 2000).

Biomarker testing is most performed on the diagnostic core biopsy specimen, which has advantages including rapid tissue fixation and the ability to use the results for systemic therapy planning, including administration of neoadjuvant systemic therapy, which is equivalent to adjuvant administration, which may allow for less extensive surgery and also enables in vivo observation of response to treatment (Van et al. 2009; Fisher et al. 1998).

Breast cancer classification and management are guided by its immunohistochemical (IHC) and molecular subtype, where prognostic information can be determined and response to therapy predicted (Sorlie et al. 2001; Perou et al. 2000). Tissue-based biomarkers, including the expression of ER, PR, and HER2, have been integral in the subtyping of tumors, prognostication, and choice of systemic therapies.

The clinical categorization of breast cancer hinges on the presence of transmembrane receptors—specifically, estrogen and progesterone receptors—alongside the amplification or excessive expression of the HER2 protein/oncogene (Wolff 2013; Hammond 2010). HER2 constitutes a tumor-associated antigen (TAA) that is amplified or overexpressed in approximately a quarter of breast cancer patients, and its presence is linked to unfavorable clinical outcomes when not suitably managed with HER2-targeted treatments (Slamon 1987).

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Given that breast cancer is a global challenge, it's imperative 2 to prioritize reducing worldwide disparities in access to diagnosis, comprehensive treatment, and innovative medications.

This consensus declaration revises and modernizes the recommendations for biomarkers employed in the diagnosis and management of breast cancer. It remains crucial to ascertain the ER and HER2 status in every instance of breast cancer prognosis, thereby establishing therapeutic avenues that encompass hormone therapy, chemotherapy, and anti-HER2 treatment.

Breast cancer possesses the potential to metastasize when malignant cells infiltrate the bloodstream or the lymphatic systemand subsequently travelto distant areas within the body. The lymphatic systemfunctions as an integral component of the body's immune defense mechanism. It consists of interconnected lymph nodes, small bean-sized glands, vessels, and organs collaborating to gather and transport transparent lymph fluid throughout body tissues, ultimately delivering it to the bloodstream (Jagsi et al. 2015). This lymph fluid coursing through the lymph vessels carries waste materials, by-products from tissues, and immune system cells.

Within the context of breast cancer, cancerous cells have the capacity to infiltrate these lymph vessels and take root within lymph nodes. Most lymph vessels from the breast converge in lymph nodes situated beneath the arm (axillary lymph nodes), within the chest near the breastbone (internal mammary lymph nodes), and around the collarbone (supraclavicular [above the collarbone] and infraclavicular [below the collarbone] lymph nodes).

When cancer cells have disseminated to the lymph nodes, the likelihood increases that these cells may have exploited the lymphatic system to spread (metastasize) to other regions of the body. Nonetheless, it's important to note that not all women with cancer cells present in their lymph nodes will develop metastases, while some women without cancer cells in their lymph nodes might encounter metastases at a later juncture.

Molecular Signatures in Breast Cancer

When addressing and validating clinically significant biomarkers, it is crucial to differentiate among three distinct categories: prognostic, predictive, and pharmacodynamic biomarkers. Prognostic markers delve into the disease's underlying biology, aiding in the assessment of potential outcomes—whether favorable or unfavorable (National Institutes of Health (US): Bethesda, MD, USA 2017). Predictive biomarkers play a role in determining effective therapies for individual patients. Lastly, pharmacodynamic/response biomarkers signify biological reactions that manifest in individuals exposed to medical interventions. While these biomarker categories are distinct, they frequently share common features. For example, an ER+ status not only suggests a positive prognosis but also predicts responsiveness to endocrine treatments.

While conventional biomarkers have proven their worth in the context of breast cancer, the disease's substantial heterogeneity and polyclonal nature challenge the capability of tissue-based biomarkers to comprehensively capture the spatial and temporal changes within a tumor as it interacts with diverse treatments. Additionally, the feasibility of sequential tissue-based biopsies for biomarker evaluation faces obstacles due to invasiveness and potential sampling errors.

Integrating alternative approaches, such as the analysis of circulating tumor DNA (ctDNA) for molecular biomarker detection and gene signatures, complements conventional methods. These approaches enable non-invasive evaluation of the disease and facilitate the examination of multiple time points during treatment, providing insights into the evolution of the tumor and its response to therapy (Ulaner et al. 2016).

Expression of ER-α stands as a favorable prognostic element and holds significant predictive value for the response to hormone therapy (Manni et al. 1980). Roughly 30–40% of individuals with advanced breast cancer expressing ER exhibit an objective response to hormone-based treatment, and an additional 20% experience disease stabilization. Furthermore, the established impact of hormone therapy on overall and disease-free survival in patients with early-stage ER-expressing breast cancer is well-documented (Dowsett et al., 2015). Notably non-toxic, hormone therapy's enduring clinical efficacy justifies its administration in patients with mammary tumors expressing ER.

The methodology for testing ER can be cost-effectively applied to fixed, paraffin-embedded tissue, making it widely accessible in most Pathology Departments. Microscopic assessment of tissue allows for the evaluation of positive reactions solely in tumor cells, circumventing challenges related to low cell density or the inclusion of normal breast tissue within the tumor growth. Comprehensive guidelines detailing approaches for immunohistochemical analysis of ERs and PRs are readily available (College of American Pathologists 2016; Hammond et al. 2010).

Typically, 70–75% of invasive breast carcinomas manifest ER-alpha expression within the nucleus. The staining intensity and the proportion of positive cells can exhibit variations, with consideration for the morphological context being essential. In cases seemingly negative for certain distinct histological types—like tubular, mucinous, or lobular carcinoma—or histological grade I, it's prudent to verify the results. The threshold for defining a positive outcome is typically set at ≥1% of positively stained nuclei, irrespective of staining intensity. The reported outcomes should specify the antibody clone utilized, along with the percentage of positive cells. Alternatively, a scoring system, such as the one introduced by Allred et al. , which combines the estimated nuclear positivity rate in cancer cells (score 0–5, based on percentage) with staining intensity (intensity 0–3), can be employed (Allred 2010). Testing for ER-α in ductal carcinoma in situ is also valuable, as hormone suppression treatment can diminish recurrence risk by 50% in patients expressing this receptor.

Along with hormone receptors, HER2 is the most important prognostic and predictive marker in breast cancer. Since the early studies by Slamon in 1987, it has been known that breast cancers that overexpress HER2 represent a highly aggressive biological subtype (Slamon et al. 1987). However, the 1998 approval of trastuzumab for therapeutic use changed the outcome in these patients, whose clinical course improved very significantly. The introduction of new targeted anti-HER2 therapies, such as lapatinib, pertuzumab, and trastuzumab emtansine (T-DM1), the last one administered with no requirement for simultaneous cytostatic, underlines the importance of identifying patients with HER2-positive breast cancer.

Any invasive breast carcinoma should be tested for HER2 overexpression, along with ERs and Ki-67. Various guidelines conclude that any HER2 test method is valid, provided the technology is standardized according to the manufacturer’s instructions and supported by an external quality-control program. This tends to be routine practice in pathology laboratories (Wolff et al. 2013; Palacios et al. 2009).

To ensure high-quality testing, it is very important for the number of technicians who perform the test, and especially the number of pathologists who interpret the results, to be as low as possible (Palacios et al. 2009). Immunohistochemistry is the most widely used technique for HER2 expression status analysis. Not only is it available in all pathology laboratories, but also it allows the sample to be assessed cheaply, simply, and

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quickly. In addition, it provides an overview of the sample, permitting easy identification of possible small positive foci in heterogeneous cases.

The human epidermal growth factor receptor 2 (HER2/neu) gene, localized on chromosome 17, encodes a 185 kDa, transmembrane member of the tyrosine kinase epidermal growth factor receptors, which are normally expressed at low levels in all epithelial cells in normal fetal and adult tissues but are also essential for cancer proliferation and survival (Press et al. 1990). HER2 gene amplification has been associated with increased levels of expression of HER2 mRNA and protein product, which lead to oncogenic signaling and resultant self-sufficiency in growth signals, uncontrolled proliferation, sustained angiogenesis, survival, enhanced invasion, and metastasis processes, which are drivers of carcinogenesis (Yarden and Pines 2012; Slamon et al. 1989). The HER2/neu gene results amplified in a variable percentage of the breast (Slamon et al. 1989; Slamon et al. 1987), ovarian (Slamon et al. 1989), bladder, endometrial (Saffari et al. 1995), salivary gland (Press et al. 1994), and gastric cancer (Park et al. 1989).

The HER family encompasses four members: EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3, and HER4/ErbB4. These receptors possess a structure comprising a ligand-binding extracellular domain, a transmembrane domain, and a cytoplasmic catalytic kinase domain, driving signaling pathways like PI3K/Akt/mTOR and RAS/RAF/MEK/ERK (Sorkin and Goh, 2008). Heterodimers of HER are more potent signal transducers than homodimers. HER2 is a primary partner for dimerization, linked to breast cancer progression and poor prognosis upon overexpression. The HER2-HER3 heterodimer is a potent oncogenic combination in breast cancer. HER2 overexpression leads to ligand-independent complex formation and kinase activity, resistant to trastuzumab through PI3K inhibition, PI3K mutations, and PTEN loss. Insulin-like growth factor receptor 1 (IGF-1R) upregulation sustains PI3K/Akt activation, leading to resistance to anti-hormonal and HER2-targeted therapies.

HER2 amplification occurs in 15–30% of breast cancers, correlating with poor prognosis and responsiveness to HER2-targeted therapies (Freudenberg. 2009). Trastuzumab, a well-known monoclonal antibody against HER2, enhances outcomes in early-stage and metastatic breast cancer (Slamon et al. 2011). Other HER2-targeted agents, including lapatinib, pertuzumab, and ado-trastuzumab emtansine (T-DM1), improve outcomes in HER2-positive metastatic breast cancer (Verma et al. 2012; Baselga et al. 2012; Geyer et al. 2006).

HER2 positivity may influence sensitivity or resistance to certain chemotherapeutic agents. For instance, HER2 positivity correlates with better responses to anthracycline-containing regimens due to co-amplification with topoisomerase II, the target of anthracyclines. However, combining trastuzumab and anthracycline raises cardiotoxicity concerns. Conversely, correlations between HER2 positivity and responsiveness to paclitaxel-containing chemotherapy remain inconclusive (Gennari et al. 2008; Hayes et al. 2007).

Breast Cancer Diagnosis

Women encountering breast symptoms or changes, like a lump, localized discomfort, nipple variations, or skin alterations, necessitate thorough diagnostic evaluation. This applies equally to women undergoing further testing following positive results on screening mammography. The diagnosis of breast cancer is established through a

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triple test approach involving clinical examination, imaging (typically mammography and/or ultrasonography), and needle biopsy (Irwig et al. 2002).

Assessment involves the comprehensive implementation of the triple test components, tailored to the patient's characteristics and presentation, ideally preceding the initiation of treatment. Accurate assessment aids in effectively distinguishing between individuals with breast cancer, benign conditions (e. g. , fibro-adenoma), or normal breast changes. This, in turn, permits reassurance or appropriate management through follow-up, potentially eliminating the need for surgical intervention.

Ultrasonography holds almost universal utility for evaluating localized symptoms, serving as an initial imaging method for young women and facilitating the identification and characterization of abnormalities detected through screening. It is also preferred for imaging-guided percutaneous biopsies. Additionally, breast ultrasonography is applied in characterizing and biopsying axillary lymph nodes in women suspected of breast cancer (Houssami et al. 2011). Magnetic Resonance Imaging(MRI) is incorporated into the imaging assessment, particularly in cases where conventional tests yield equivocal, inconclusive, or conflicting outcomes. It is also recommended for women with breast implants and those with axillary nodal metastases but no detectable breast tumor (Sardanelli 2010; Morrow et al. 2011). While preoperative MRI is selectively employed for staging newly diagnosed disease, its clinical benefit remains debated (Sardanelli 2010). However, MRI is advised for preoperative evaluation of newly diagnosed invasive lobular cancers.

Ethical Consideration

A formal introductory letter was provided to the supervisor of the histopathology laboratory unit, AE-FUTHA 1, seeking authorization to utilize archived tissue samples for the purpose of this research. Additionally, access was requested to the histopathology register and bench books to retrieve patient information in accordance with ethical standards, upholding the utmost confidentiality of patient data. This adherence to ethical principles aligns with the ethical approval obtained from the Faculty of Health Sciences and Technology at Ebonyi State University Abakaliki

Materials and methods

Sample Collection, Preparation, and Staining

The research samples were obtained from the archives of the histopathology laboratory at AE-FUTHA in Abakaliki. The technique of formalin fixation and paraffin embedding was employed to maintain the structural integrity and cellular details of the tissue specimens. This preservation method has become the standard practice in diagnostic surgical pathology. Storing formalin-fixed, paraffin-embedded (FFPE) blocks at room temperature over the long term is more economically efficient compared to freezing tissues at ultra-low temperatures, considering factors such as maintenance, space, and labor costs. Pathology departments routinely store a large number of FFPE blocks as opposed to frozen tissues. This underutilized resource constitutes a substantial collection of tissue material accompanied by extensive clinical follow-up, thus serving as a valuable asset for translational clinical research.

Formalin-fixed paraffin-embedded blocks are derived from human tissues obtained during routine diagnostic or therapeutic procedures in hospitals. These tissues are sent to the pathology department, where they are sectioned and embedded in paraffin blocks for histopathological examination. These blocks are stored in pathology

laboratory archives for extended periods, remaining accessible for potential tests, re-examinations, and research endeavors.

A total of 134 tissue blocks, which had been preserved using paraffin wax embedding, were subjected to the microtome machine. Thin sections were meticulously extracted and subsequently immersed in 20% alcohol before being transferred to a water bath. Employing a "floating in and out" technique, the delicate sections were affixed onto pristine, grease-free glass slides and subsequently thoroughly dried using hot air to ensure secure adhesion. Following this process, the slides were fully prepared for staining; routine histology staining was carried out as described by Nnaemeka (2021)

Periodic Acid Schiff (PAS)

Periodic acid oxidizes the 1:2 glycol groups in the tissue to di-aldehydes to react with fuchsin sulfurous acid solution (Schiff's) to form a magenta compound. Deparaffinize tissue sections using xylene for 5 minutes each in 3 changes of xylene and take to 2 changes of alcohol for 5 minutes each. Bring sections to water, stain in periodic acid for 5-10 minutes and wash thoroughly in tap water and rinse in distilled water. Stain in Schiff's solution for 20 minutes, wash thoroughly in running water and Counter stain progressively in hematoxylin. Blue in Scot tap water, dehydrate, clear, and mount using DPX (Nnaemeka 2021).

The Immunohistochemistry Methods

The employed technique was the Avidin-Biotin Complex (ABC) method, also known as the Avidin biotin Immunoperoxidase method. Tissue sections, measuring 2 microns in thickness, were prepared from formalin-fixed and paraffin-embedded samples for immunohistochemistry (IHC). To retrieve antigenic sites, a citric acid solution was utilized in conjunction with a pressure cooker. Blocking of peroxidases, proteins, and biotin was conducted using Hydrogen peroxide, avidin, and biotin, respectively. Estrogen receptor and HER-2 antibodies were diluted at a ratio of 1:100 and incubated with the sections. This was followed by the application of biotinylated secondary antibodies, streptavidin, and a DAB/substrate reaction.

The estrogen receptor and HER-2 antibodies used in this study are products of Novocastra, now owned by LEICA. The antibody dilution factor applied was a 1:100 dilution for all antibody markers. The archived tissue was sectioned to 3 microns using a rotary microtome and placed on a hot plate at 70 degrees Celsius for a minimum of 1 hour. Sections underwent a series of transitions through xylene, descending grades of alcohol, and water to reach the aqueous environment. Antigen retrieval was accomplished by subjecting the sections to a citric acid solution (pH 6. 0) in a pressure cooker for 25 minutes. Subsequently, the sections were gradually cooled with water. Peroxidase blocking involved covering the sections with 3% hydrogen peroxide (H_2O_2) for 15 minutes, followed by washing with PBS. Protein blocking was achieved using avidin for 15 minutes and endogenous biotin in the tissue was blocked with biotin for an additional 15 minutes. After rinsing with PBS, the diluted primary estrogen receptor and HER-2 antibodies (1:100) were applied to the sections for 60 minutes. Excess antibodies were washed away with PBS, and a secondary antibody (LINK) was applied for 15 minutes. Subsequently, the horseradish peroxidase (HRP) enzyme (LABEL) was applied for another 15 minutes. A working DAB solution, formed by combining 1 drop (20 microns) of DAB chromogen with 1ml of DAB substrate, was then added to the sections. This solution initiated the appearance of brown reactions, especially in positive targets. After washing off excess DAB solution and precipitates with water, sections were

counterstained with Hematoxylin for a minimum of 2 minutes, followed by a brief bluing process. Sections underwent dehydration in alcohol, clearing in xylene, and were ultimately mounted in DPX.

Results

Over the span of 2019 to 2021, a total of 73 breast tumor samples were diagnosed at AEFUTHA, constituting 54. 5% of the breast tumor samples submitted for assessment (as indicated in Table ii). Specifically, there were 45 (33. 6%) breast cancer diagnoses in 2019, followed by 50 (37. 3%) in 2020, and 39 (29. 1%) in 2021.

Visual representations of breast tissue samples stained with H&E, PAS, and IHC can be observed in plates 1 to 8. These stained sections predominantly revealed characteristics indicative of ductal carcinoma, colloid carcinoma, and Paget disease of invasive ductal carcinoma.

The immunohistochemistry results were assigned a grading ranging from 0 to 5 based on intensity. Among the 62 breast cancer tissue sections, 59 (95. 2%) exhibited negative ER expression, whereas 3 (4. 8%) displayed very weak expression. In contrast, HER2 demonstrated moderate expression in 50 (80. 6%) of the samples, strong expression in 7 (11. 3%), weak expression in 4 (6. 5%), and very weak expression in 1 (1. 6%). Further details are provided in table 3

Table i Age demographics associated with breast tissue samples processed at AEFUTHA from 2019 to 2021

Age	**Year**			**Total**
	2019	**2020**	**2021**	
15–24	1	0	0	1
25–34	5	7	5	17
35–44	7	8	6	21
45–54	3	7	7	17
55–64	12	9	6	27
65–74	6	5	5	16
75–84	3	10	9	22
85–94	5	2	1	8
95–104	3	2	0	5
Total	45	50	39	134

Table ii Classification of breast tissue samples processed at AEFUTHA from 2019 to 2021 based on tumor type

Tumor type	**Year**			**Total (%)**
	2019	**2020**	**2021**	
Benign	22	22	17	61 (45.5)

Malignant	23	28	22	73 (54.5)
Total (%)	45 (33.6)	50 (37.3)	39 (29.1)	134 (100)

Table iii: Immunohistochemical expressions of ER and HER2 in breast cancer tissue samples processed at AEFUTHA between 2019 and 2021 (n = 62)

Immunostaining reaction grading	**ER**	**HER2**
Negative (0/5)	59 (95.2%)	0
Very weak (1/5)	3 (4.8%)	1 (1.6%)
Weak (2/5)	0	4 (6.5%)
Moderate (3/5)	0	50 (80.6%)
Strong (4/5)	0	7 (11.3%)
Very strong (5/5)	0	0

Fig. 1

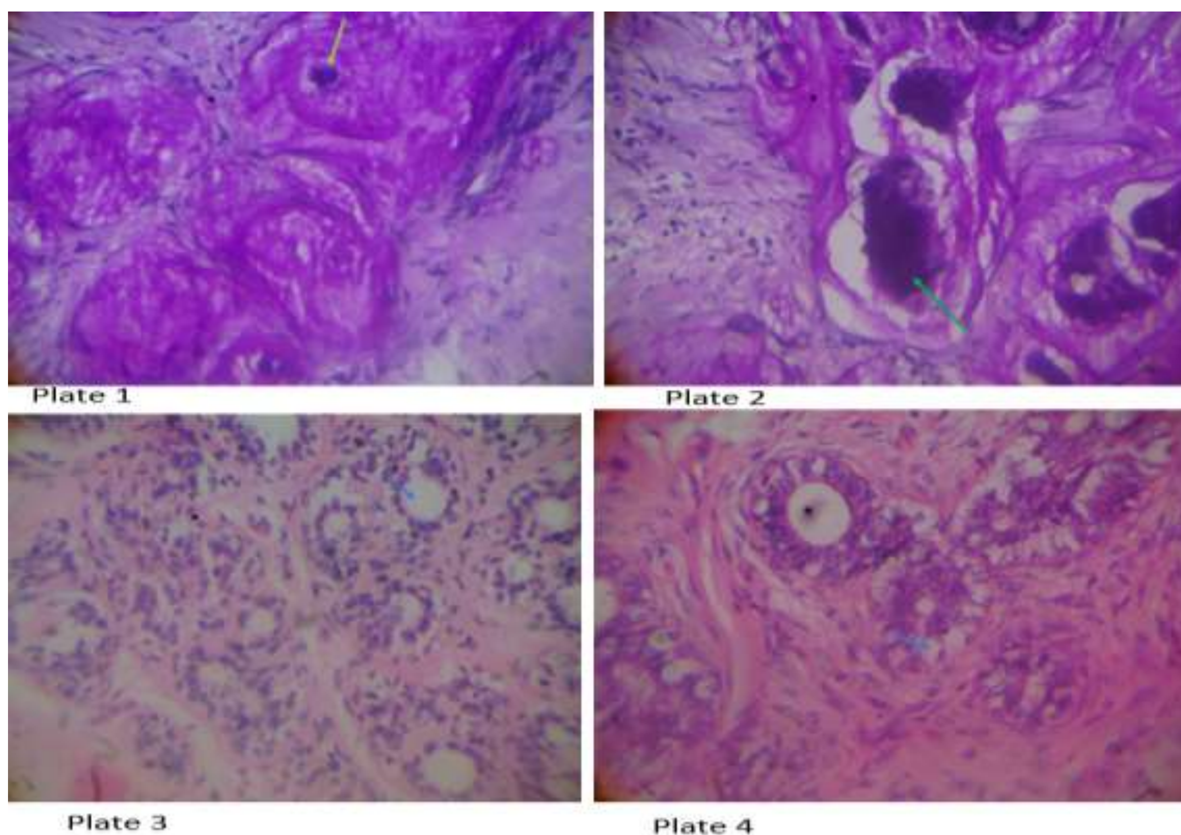

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Plate 1 (Magnification X200) illustrates breast tissue stained with PAS, revealing an evident ductal proliferation, along with ductal calcification (indicated by the green arrow), and a cribriform pattern characteristic of ductal carcinoma. In Plate 2 (Magnification X200), breast tissue stained with PAS showcases copious, bluish-stained mucin. Suspended within the mucin are malignant cells, defining the condition as colloid carcinoma. Plate 3 (Magnification X200) displays breast tissue highlighting nucleoli, hyperchromatic nuclei, infiltrating malignant cells extending beyond the stroma, and distinctive cookie-cutter spaces. These findings are indicative of invasive ductal carcinoma. Plate 4 (Magnification X200) portrays breast tissue featuring nucleoli, hyperchromatic nuclei, and an abundance of clear cytoplasm, all consistent with Paget's disease associated with invasive ductal carcinoma.

Fig. 2

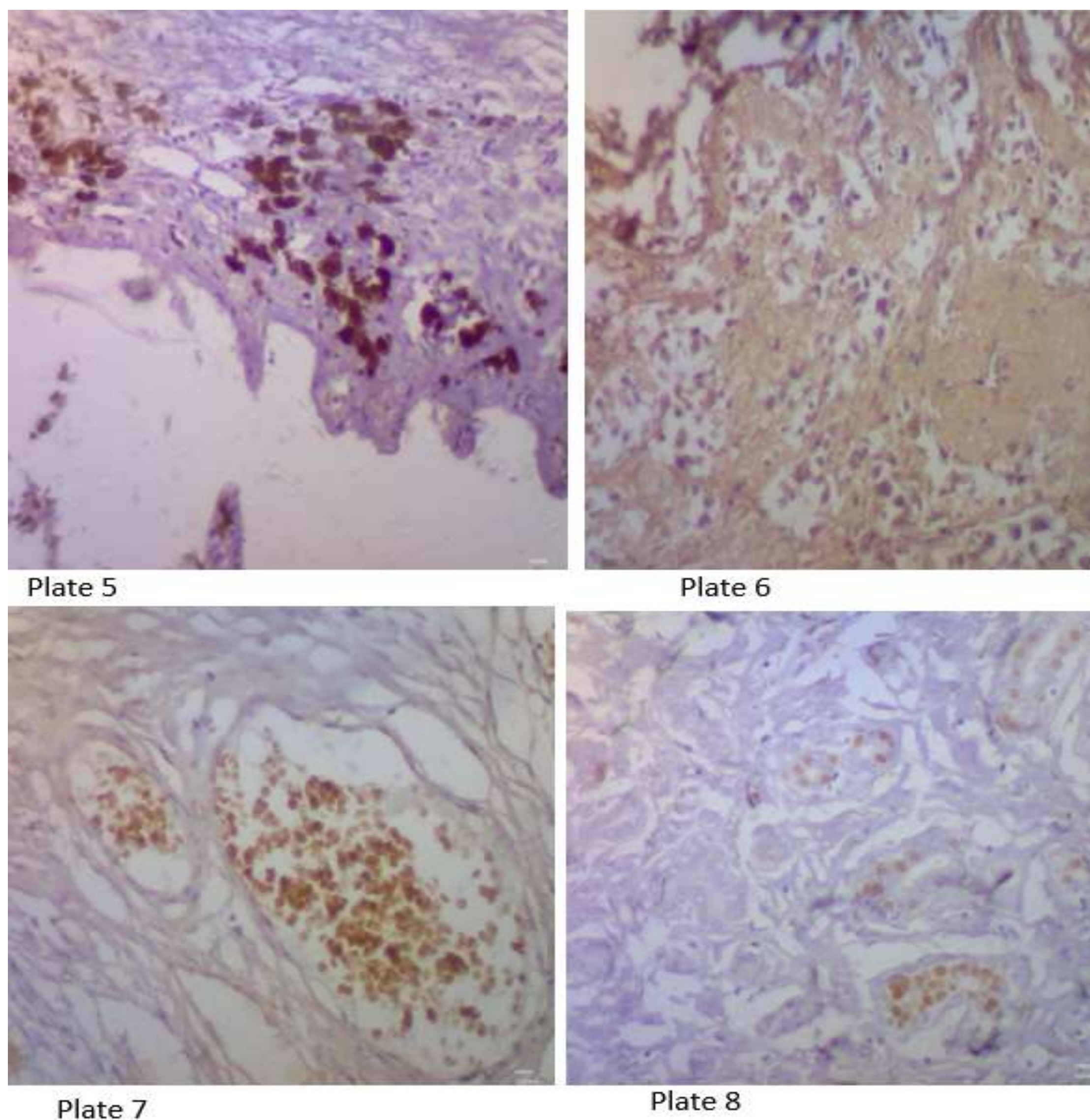

Plate 5 (Magnification X200) exhibits breast tissue subjected to ER Antibody staining. The immunohistochemical assessment yields a grade of 4 out of 5. In Plate 6 (Magnification X200), breast cancer tissue is treated with HER-2 Antibody, resulting in an immunohistochemical rating of 3 out of 5. Plate 7 (Magnification X200) showcases benign breast tissue exposed to HER-2 Antibody staining, resulting in an

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immunohistochemical grade of 0 out of 5. Plate 8 (Magnification X200) presents breast cancer tissue subjected to ER Antibody staining, resulting in an immunohistochemical grade of 0 out of 5.

Discussion

Breast cancer ranks as the most diagnosed malignancy on a global scale (Wild et al. 2020), underlining the significance of early detection to mitigate its impact on health and mortality, especially within asymptomatic populations (Tse et al. 2008). Among the key factors evaluated for breast cancer diagnosis, the presence of calcification within breast tissues holds notable importance (Tse et al. 2008). This study observes ductal calcification and a cribriform pattern in the examined breast cancer tissue sections, characteristic of ductal carcinoma. Calcification's pivotal role in diagnosing ductal carcinoma in situ, a precursor stage of breast cancer, is well-established (Tse et al. 2008), with an incidence rate ranging from 42% to 72% (Yang and Tse 2004).

The study also uncovers instances of colloid breast carcinoma, a rare variant constituting 2% of all breast carcinomas (Lei et al. 2016). Moreover, Paget's disease is a noteworthy discovery, with select breast cancer tissue sections displaying nucleoli, hyperchromatic nuclei, and abundant clear cytoplasm consistent with invasive ductal carcinoma linked to Paget's disease, accounting for 1% to 4. 3% of all breast carcinomas (Sakorafas et al. 2001). Notably, Paget's disease often accompanies underlying ductal carcinoma in situ and/or invasive ductal cancer (Sakorafas et al. 2001).

This study's central focus lies in the comparative analysis of immunohistochemical expression of ER and HER2 biomarkers in breast cancer tissues. ER serves as a prevalent biomarker for breast cancer, manifested across the cell membrane, cytoplasm, mitochondrion, and nucleus (Yu et al. 2021). Over 70% of breast cancers classify as estrogen receptor-positive, based on immunohistochemical detection of ER expression in at least 1% of tumor cells (Scabia et al. 2022).

Surprisingly, the study reveals robust estrogen receptor expression in benign breast tissue (see plate 5), contrasting with a predominantly negative expression of estrogen receptors in the majority of breast cancer tissues (see plate 7 and table 3). However, according to Collins et al. (2005), breast cancers tend to exhibit either strongly positive ER staining or a complete lack thereof. The prevalence of low-positive ER tumors generally ranges from 3% to 9% (Yi et al. 2014). Loss of ER expression might result from extended cold ischemic time (Nkoy et al. 2010) or diminished tissue quality (Neumeister et al. 2014), with mechanisms such as genetic changes and epigenetic modulation contributing to ER expression loss during cancer progression.

Hormone receptor testing is pivotal in guiding treatment decisions for breast cancer patients, as those with ER-positive tumors exhibit significant responses to endocrine therapy (Yu et al. 2021). ER status significantly influences clinical decisions and outcome predictions for invasive breast cancer patients (Regan et al. 2006). ER-positive tumors are eligible for endocrine therapy, whereas ER-negative tumors often necessitate chemotherapy, resulting in poorer outcomes (Zhang et al. 2020).

ER-α and HER2 represent key biomarkers distinguishing distinct breast cancer subgroups (Pinhal et al. 2012). Notably, tumors with low or negative ER expression are more likely to express HER2 (Yu et al. 2021). Meta-analysis data indicate HER2 positivity ranging from 10% to 14% in ER-high tumors, escalating to 24% to 28% in ER-low tumors (Yu et al. 2021). HER2's significance extends to targeted therapy efficacy and prognostic

predictions (Tomiguchi et al. 2016), as it is overexpressed in 20% to 30% of breast cancers, often signifying aggressive disease, higher recurrence rates, and increased mortality (Mitri et al. 2012; Hudis, 2007).

In this recent study, a substantial portion of cancer tissue samples exhibited moderate HER2 expression, while the majority lacked ER expression. This aligns with Tomiguchi et al. (2016) that ER and HER2 positivity inversely correlate, with ER expression being quantitatively higher in HER2-negative tumors. Conversely, Pinhal et al. (2012) found a positive correlation between ER and HER2 at both protein and RNA levels, albeit in HER2-negative tumors. The present study underscores the prominence of HER2 status as a key discriminator.

Conclusion

In the ongoing battle against the devastating impact of breast cancer, significant strides have been taken in the realms of diagnosis and treatment. Much like other cancer types, early detection is pivotal in securing a favorable prognosis for breast cancer patients. This endeavor has led us to delve into the comparative assessment of ER and HER2 immunohistochemical expressions as diagnostic tools for breast cancer. Our study has illuminated a noteworthy pattern: the breast cancer tissues under scrutiny exhibited elevated HER2 expression alongside diminished ER expression, indicating a clear inverse relationship between these markers. This consequential finding is poised to contribute substantially to the realms of diagnosis, prognosis monitoring, and the formulation of targeted therapeutic strategies for breast cancer patients.

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Authors contributions

Uzoigwe D. C. Researcher: Conceptualization, methodology design, data collection, selection of archived tissue blocks from the tissue bank and staining. She also contributed significantly to the writing of the introduction and methods sections of the manuscript.

Ewa I.O: Critical review of the manuscript. Onyekachi provided expertise in the experimental design and contributed to the revision of the results, discussion sections and formatting of the manuscript to reflect journal guidelines on referencing.

Olisa A. G: Statistical analysis and interpretation of data. Olisa was responsible for generating the tables used in the results section.

Idakari C. N: Writing, editing, and formatting of the manuscript. Idakari contributed to every section of the paper, ensuring clarity and coherence in the overall narrative.

Ibe U.C: Final review and proofreading of the manuscript before submission. Ibe ensured the accuracy of references, adherence to journal formatting guidelines, and photomicrograph plates reporting quality and standardization.

Finan U.F: Finan re-examine the sorted tissue formalin fixed processed blocks to ensure corresponding record from the patients record book, and she also proofread the manuscript before submission.

Akanni B. A: Akanni B. A. played an active role in formulating the methodology and was responsible for drafting the schematic workflow during the laboratory work.

Joseph C. U.: Joseph C. U. was responsible for the gross examination of tissues that had not yet undergone processing but met the criteria for inclusion in this study. This examination was conducted by Joseph and was subsequently incorporated following routine staining and microscopy reports.

Okparaoka S.U.: Okparaoka assumed a supervisory role at various laboratory workbenches and served as a quality control analyst throughout the entire duration of this study.

Ude U.A: She oversaw the staining protocols and the subsequent transfer of slides for microscopy. Additionally, she actively participated in the manuscript review process alongside the authors.

Okorie N. Supervisor: Oversight and guidance throughout the research process. Okorie provided valuable insights during the research design phase and reviewed the manuscript at multiple stages. He developed the standard operating procedure manual for microtomy, staining, photomicrograph and slide reports offering constructive feedback for improvement.

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Data Avaliability

We understand the importance of data availability for ensuring transparency, reproducibility, and the overall scientific integrity of our work. By making these resources accessible as presented in tables and photomicrographs, we aim to facilitate further investigation and collaboration within the scientific community. Please let us know if there are any specific requirements or guidelines regarding data availability that we should address or any additional information you may need from us. We appreciate your commitment to maintaining high standards of scientific rigor and transparency.

Declarations

We recognize the importance of transparently disclosing any potential conflicts of interest to maintain the integrity and credibility of the research and the publication process. we assure you that we acted by following per the guidelines and standards set forth by the journal of Cancer Research and Clinical Oncology regarding the declaration of interest.

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